said hinge region being a cysteine-free flexible amino acid sequence not normally associated with said leader sequence and comprising at least two amino acids which promote cleavage by said cleavage agent at said cleavage site, and

a second sequence of amino acids linked to said first sequence and defining a selected target polypeptide, whereby said cleavage site is a favored site for cleavage upon treatment of said fused polypeptide with said cleavage agent when said fused polypeptide is disposed in solution and when said amino acid sequence defining said target [fused] polypeptide is disposed in its tertiary conformation.

D' cont

R E M A R K S

The invention features a fused polypeptide, produced by expression of a recombinant DNA, comprising a leader sequence, a hinge region which is a cysteine-free flexible amino acid sequence not normally associated with said leader sequence and comprises at least two amino acids which promote cleavage at a cleavage site positioned at the carboxy terminal amino acid(s) of the hinge, and a target polypeptide which is covalently linked to the leader sequence via the cleavage site within the hinge region. When the fused polypeptide is disposed in solution and when the amino acid sequence defining the target polypeptide is disposed in its tertiary conformation, the cleavage site of the fused polypeptide is a favored site for cleavage upon treatment of a cleavage agent.

Claim 27 has been amended to better define the invention by requiring that the cysteine-free hinge be flexible and be a sequence which is not normally associated with the leader sequence. In addition, as amended, claim 27 now requires that the cleavage site be the carboxy terminal amino acids of the hinge region. Thus, cleavage at the cleavage site releases a target polypeptide consisting of only native amino acids.

The claims were previously rejected under 35 U.S.C. §103 over Cousens et al., U.S. Patent 4,741,180. Already of record in the parent application of the instant file-wrapper-continuation application is a Rule 1.131 affidavit which effectively removes the Cousens et al. continuation-in-part application, filed March 28, 1986, as prior art to the instant application.

The Cousens et al. parent disclosure does not anticipate then claimed invention under 35 U.S.C. §102 in the amended claims because there is no disclosure in the Cousens et al. parent application of a fusion protein containing a hinge region in which the cleavage site is the carboxy terminal amino acid(s) of the hinge. Claim 1, as amended, covers a fusion polypeptide comprising a leader sequence fused to a target polypeptide through a hinge region which contains a carboxy terminal cleavage site.

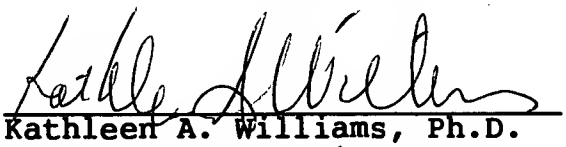
The Cousens et al. parent application does not render the instant claims obvious under 35 U.S.C. §103, as there is no suggestion in the Cousens et al. parent disclosure of an easily-cleavable fusion protein, i.e., that a hinge region and cleavage site may be engineered between the leader sequence and target polypeptide of a fusion protein so as to give preferential cleavage of the hinge region cleavage site.

Applicants have provided a solution to a long-recognized problem, i.e., how to cleave a fusion protein easily and preferentially to produce a target polypeptide in its native form, which may even contain identical cleavage sites within its sequence. The invention provides for a flexible cysteine-free hinge region with carboxy terminal amino acids (i.e., the amino acids directly adjoining the amino terminus of the target polypeptide) which are preferentially susceptible to cleavage when the fusion protein is in solution and the target polypeptide is disposed in its tertiary conformation.

BEST AVAILABLE COPY

Applicants submit that all the claims are in condition for immediate allowance, and such action is respectfully requested.

Respectfully submitted,



Kathleen A. Williams, Ph.D.
Attorney for Applicants
Reg. No. 34,380

Date: February 3, 1993
Testa Hurwitz & Thibeault
53 State Street
Boston, MA 02109
617-248-7100
189kam2054/6.AV5